BioPAL *New ways to make measurements in the life sciences.*
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Application Note 8.

Title

Gadoluminate™ (**CL-20P02-2)**, an ultrasmall gadolinium oxide colloid for MRI and MRA imaging CPR/DEV/EVG

Keywords

Gadoluminate, MRI, MRA, T1, nanoparticle, gadolinium oxide, colloid, vascular imaging, BioPAL

Summary

BioPAL is pleased to introduce ***Gado***luminate, the first commercially available gadolinium nanocolloid for MRI and MRA applications. The reagent, an ultra-small gadolinium oxide colloid (30 nm), is stable in aqueous suspension under a variety of challenges including autoclaving. ***Gado***luminate provides a strong T1 brightening signal comparable to the same molar concentration of gadolinium-DTPA. This report provides general toxicology data and demonstrates its suitability as a vascular contrast reagent for magnetic resonance angiography (MRA) and for MRI applications.

Toxicology

Acute Dose Study With partial assistance from NIH SBIR program (1 R44 HL080837-01), BioPAL has evaluated the toxicity of ***Gado***luminate in a swine model (male, Yorkshire miniswine). Subjects were injected at two doses (0.01 mmol Gd/kg and 0.05 mmol Gd/kg). These doses were based on previous *in vivo* data that demonstrated that high quality MRI images are achieved at a dose of 0.01 mmol Gd/kg of ***Gado***luminate. Each animal received a single dose and was clinically observed for 7 days. All animals appeared normal during the injection and the 7-day observation period. At the end of the study, organs were weighed and tissues were prepared for histopathology. Blood was taken at necropsy for hematology and blood chemistry, and urine was collected prior to necropsy for urinalysis. No negative observations were seen at necropsy. Histopathology of the kidneys and liver also reported normal findings.

Subacute Dose Study For the subacute swine study, two dose levels (0.01 mmol Gd/kg and 0.05 mmol Gd/kg) were evaluated. Each animal received the specified dose once a day for 14 days, for a total accumulated dose of 0.14 mmol Gd/kg and 0.70 mmol Gd/kg, respectively. Animals within each dose group were observed and weighed daily. At the end of the study period, organs were weighed and tissues (liver and kidney) prepared for histopathology. Blood was also taken at necropsy for hematology and blood chemistry. All animals appeared normal during injection. No negative observations were observed at necropsy. Histopathology of the kidneys reported normal findings. Mild hepatic alterations were observed, consisting of minimal multifocal mononuclear cell infiltrates in the hepatic sinusoids for both doses studied. A minimal increase in sinusoidal Kupffer cells was reported only for the higher dose study.

Acute and subacute toxicity studies were also performed in mice and rats. The results of these studies complement the findings in swine. Overall, ***Gado***luminate possesses a favorable toxicity profile for applications in MRI and MRA research.

Pharmacokinetics

We investigated the long-term *in vivo* clearance of ***Gado***luminate in a large animal model. Yorkshire miniswine were administered the reagent by arterial injection at a dose of 0.05 mmol Gd/kg. Blood samples were collected post administration at time intervals, as follows: 1, 5, 10, 15, 30 and 60-minutes, 1, 2, 3, 6, 12, 24-hours and 2, 5, 10, 15-days. Daily fecal matter was collected. At the end of the blood sampling period, organ samples were collected (liver, heart, kidneys, lungs, spleen, stomach, small and large intestine, brain and muscle). Tissues were weighed and placed in sample vials. All samples, blood, feces, and tissues were

analyzed for gadolinium content *via* neutron activation analysis following standard procedures (BioPAL, neutron activation service).

The calculated blood half-life ($t_{1/2}$) of ***Gado***luminate in the swine model proved to be approximately 12.5 hours (Figure 1). In addition, after 15 days post administration of ***Gado***luminate, no adverse effects were observed in any subject and all organs appeared normal at necropsy consistent with the toxicity study discussed above. Neutron activation analysis of organ samples showed that gadolinium resides chiefly in the liver, spleen, and lungs with additional activity measured in the stomach and small and large intestines. Gadolinium was also detected in fecal samples. Despite the long blood half-life, there was no gadolinium in the brain, myocardium and skeletal muscle.

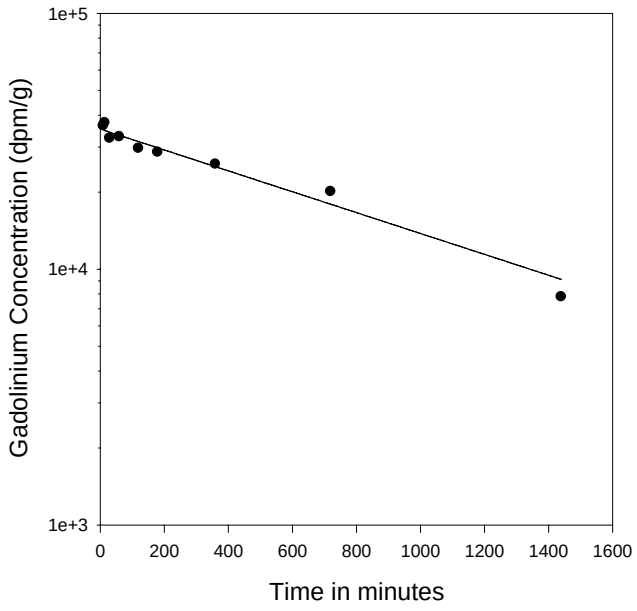

Figure 1: Blood clearance of ***Gado***luminate in swine. Each point is the average percent gadolinium in blood taken from three swine. The calculated blood half-life is ~12.5 hours.

T1 Characterization in an In Vivo Model

Cardiac MRI and T1 characterization were evaluated in a swine model. Cardiac MRI was performed on anesthetized swine while in the MRI scanner. Animals were given ascending doses of ***Gado***luminate from 0.001 up to 0.1 mmol Gd/kg. The dose was delivered *via* a power injector followed by a 10 ml saline flush. After each dose, a Modified Look-Locker Inversion Recovery sequence with 11 different inversion times was performed. The signal intensity was measured in blood and in the myocardium. The data was fitted to a T1 relaxation curve based on the 3 parameter Levenberg-Marquardt curve fitting procedure.

All animals in the study received the full range of dose levels with no adverse effect that could be attributed to ***Gado***luminate. Figure 2 shows the change in T1 signal in blood with accumulative dosage of ***Gado***luminate. Vascular contrast was observed immediately, with ample contrast of the vascular space achieved at the expected dose of 0.01 mmol Gd/kg body weight.

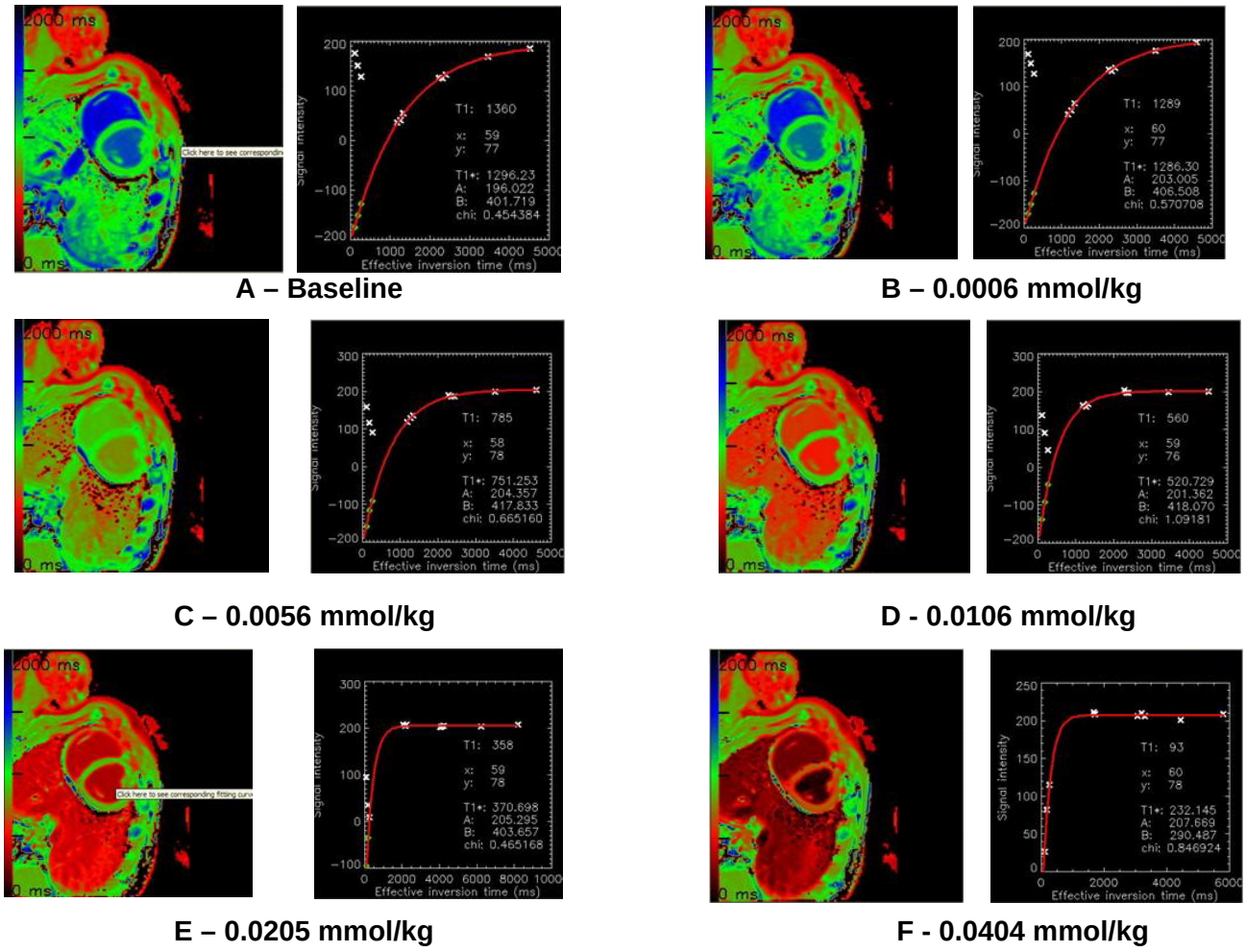

Figure 2: For one subject, MRI images and corresponding T1 relaxation curve for blood is shown for baseline (no contrast) and for accumulative doses of ***Gado***luminate.

MRI enhancement associated with ***Gado***luminate was largely limited to the vascular blood space. Tissue accumulation was minimal. For comparison, Figure 3 (next page) shows the change in T1 signal in myocardial tissue as the accumulative dose of ***Gado***luminate. The dramatic shift in T1 signal with increasing ***Gado***luminate contrast as compared to baseline is readily apparent in blood (Figure 2), but significantly less in myocardial tissue (Figure 3) showing that ***Gado***luminate is retained exclusively in the vascular compartment.

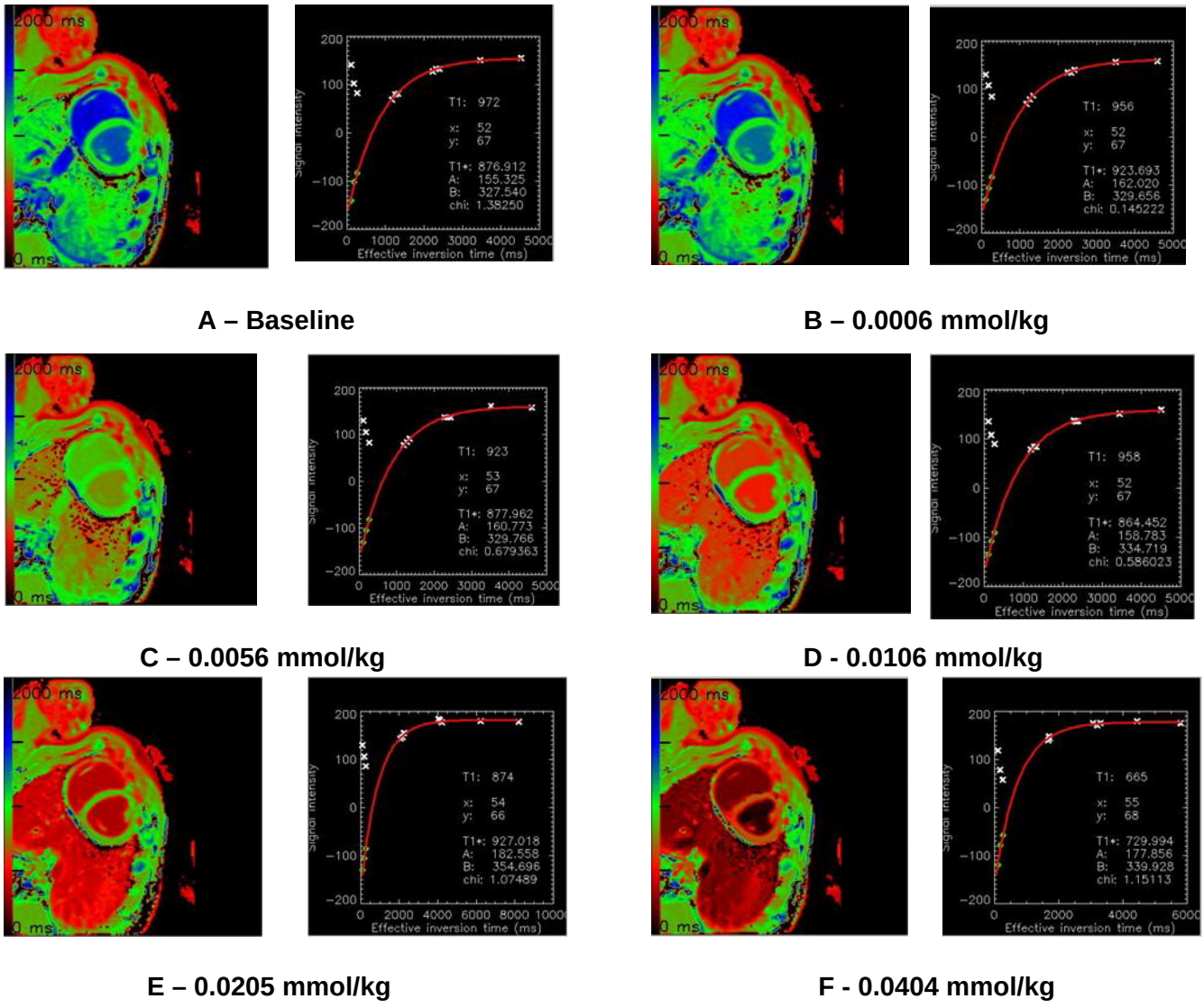

Figure 3: For the same subject shown in Figure 2, MRI images and corresponding T1 relaxation curve for the myocardium is shown for baseline (no contrast) and for accumulative doses of ***Gado***luminate.

Even at an accumulated dose that is 10x greater than our recommended starting dose (0.01 mmol Gd/kg), ***Gado***luminate was still largely restricted to the vascular space with limited tissue contamination (Figure 4). The total accumulative dose of 0.1 mmol Gd/kg was achieved over a time period of approximately 45 minutes. This time is sufficient to allow low molecular weight contrast agents, like Gd-DTPA, to diffuse into the tissue space and thereby corrupt the MRA image. However, this was not observed with ***Gado***luminate.

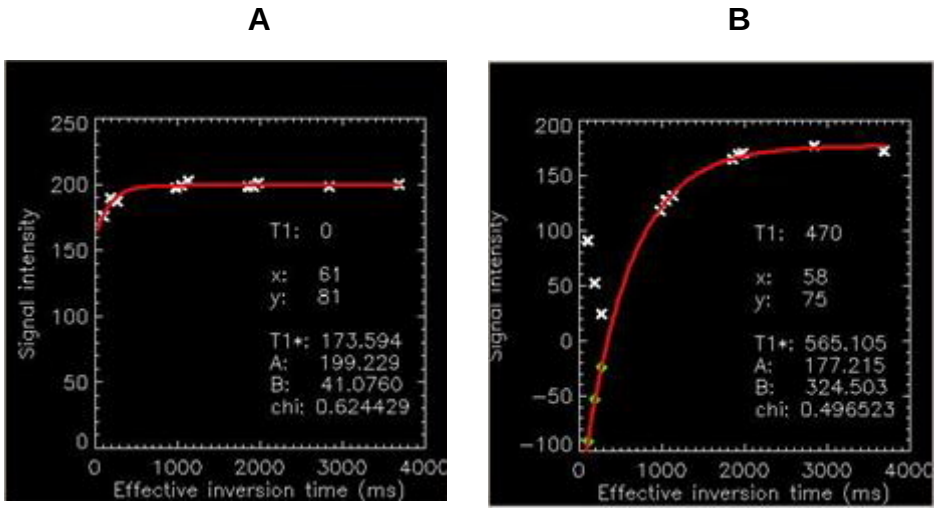

Figure 4: At an accumulated dose of 0.101 mmol Gd/kg, the vascular blood space has achieved maximum contrast (**A**), as compared to myocardial tissue (**B**). This image was acquired approximately 45 minutes after the start of the dosing. As a result, much of the contrast agent (***Gado***luminate) had been in circulation for a substantial period of time.

Efficacy Studies in Normal Swine

This study was designed to compare our MRA enhancing reagent to conventional Gd-DTPA MRA. Free-breathing and breath-hold MRA was performed in a swine model 1) without the use of a contrast reagent; 2) with ***Gado***luminate; and 3) with an extravascular enhancing reagent (Gd-DTPA). Animals were placed under anesthesia and vital signs monitored. A catheter was then placed to allow for contrast reagent administration. Gd-DTPA evaluation was first performed on all animals. Images were acquired using free-breathing MR sequence and then using a breath-hold MR sequence, first without the aid of contrast (standard time-of-flight protocol) and then with the addition of Gd-DTPA. The animals returned to the MRI suite one week later for evaluation of ***Gado***luminate and the MRI protocol was repeated.

There is a striking difference in the degree of contrast enhancement achieved by ***Gado***luminate as compared to the comparable dose of Gd-DTPA. Poor contrast was provided by Gd-DTPA largely due to the Gd-DTPA diffusing into the tissue space and thereby reducing MRA contrast. The colloidal ***Gado***luminate was retained in the blood stream providing a higher degree of contrast compared to a comparable dose of Gd-DTPA (Figure 5).

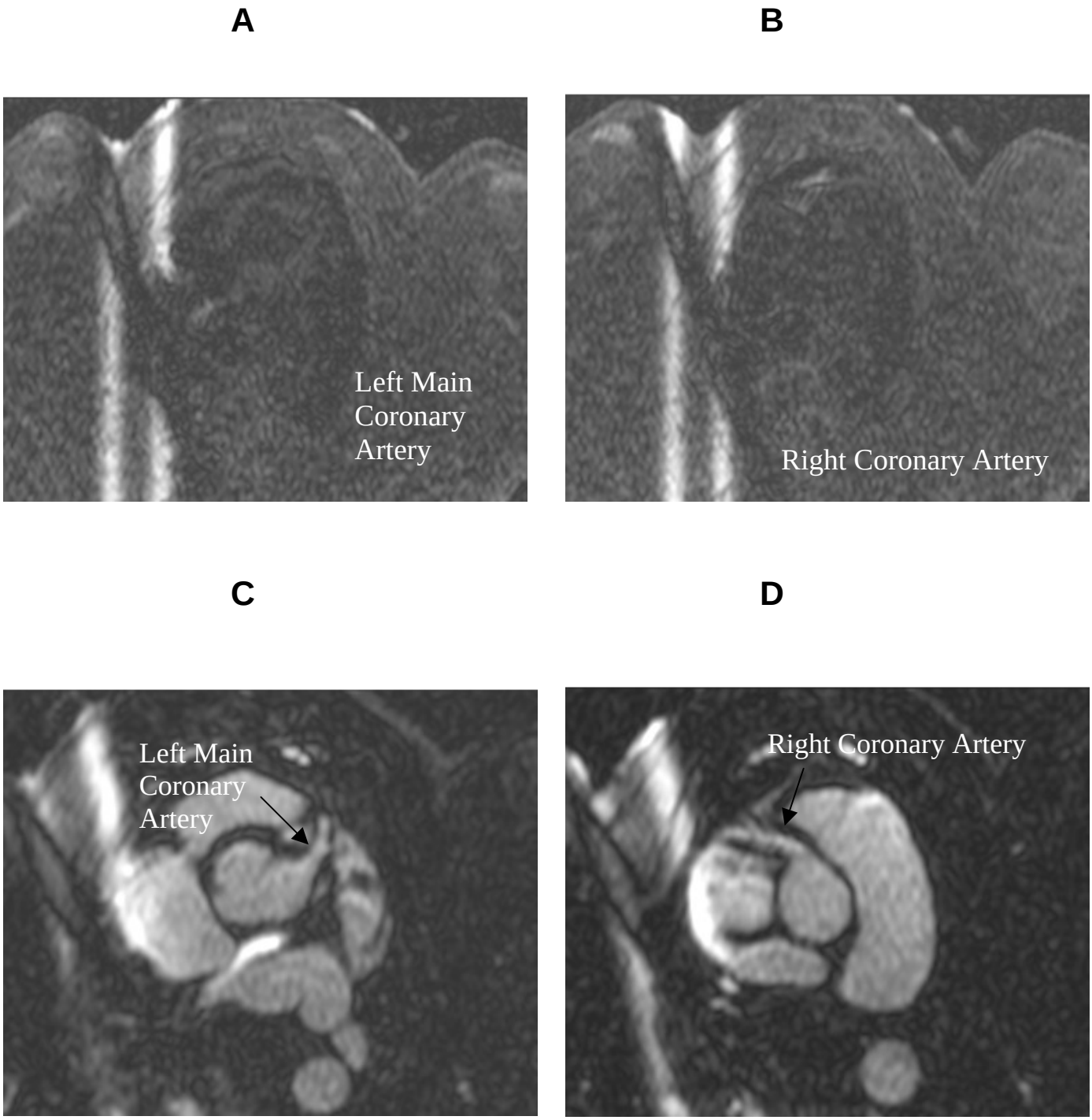

Figure 5: A-B) MRA following Gd-DTPA injection and C-D) MRA following ***Gado***luminate injection. ***Gado***luminate provided strong contrast that allowed visualization of the left and right main coronary artery. Under the same conditions, Gd-DTPA provided lower contrast than ***Gado***luminate.